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In vitro Culture and Grafting of White Pine Shoot Apical Meristems.

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Introduction

The research work described in this report has been supported by funds from the Minnesota Landscape Arboretum Land Grant Chair Endowment. Funds have also been provided by the U.S. Forest Service and the Eastern Region of the International Plant Propagator's Society. The long-term research goal of this project is to develop a vegetative propagation method for mature white pine (*Pinus strobus* L.) clones. Unfortunately, rooting of cuttings of white pine, like most other conifers, decreases with increasing plant age. Rejuvenation, that is restoration of rootability (and other juvenile characteristics) to mature tissues, may be possible by culturing or grafting shoot apical meristems in vitro. Rejuvenated shoots have been obtained via meristem culture from giant sequoia, *Sequoiadendron giganteum* (Monteuuis, 1991) and via meristem grafting from maritime pine, *Pinus*

pinaster (Dumas et al, 1989). If rejuvenated shoots can be obtained from mature white pines, they will be used as a stock block source of rootable cuttings to clonally propagate superior genotypes. In vitro culture and grafting of pine meristems may also have utility for obtaining "pathogen-free" plant materials and/or as a research method for investigating factors that influence bud dormancy and growth. In this report we describe progress in developing methods for in vitro culturing and grafting of white pine shoot apical meristems.

Excised Meristem Culture

Experiments have been conducted with juvenile (from 4-6 week-old seedlings) and mature (from 90-year old trees) meristems cultured in vitro. The apical dome and 4-6 closely appressed leaf primordia are excised and placed on half-strength agar solidified Schenk & Hildebrand medium in 3 cm plastic petri dishes. Meristems are cultured under low irradiance fluorescent light at 26C. Several culture factors have been tested to optimize meristem growth. The most important culture factor identified to date is insertion of a piece of a cellulose acetate filter between the excised meristem and the culture medium (Romberger, 1970). Use of these filters dramatically improved the survival of mature meristems and growth of juvenile meristems with juvenile meristems developing into shoots with many primary needles. In a few cases meristem-derived juvenile shoots formed adventitious roots and developed into plantlets. However, even with the use of cellulose acetate filters, new leaf formation from mature meristems was quite limited when meristems were excised from branches collected in spring prior to bud break and candle elongation.

After several unsuccessful attempts to stimulate mature meristem growth and development by varying light quality and irradiance and several growth regulators in the medium, we studied the effect of removing meristems from branches at different stages of bud break and candle elongation. This experiment was based on knowledge that spring is the season when meristems produce leaves for the next year's growth (Owston, 1969). Branches were collected in late March or April before bud break, but after the chilling requirement had been fulfilled, and stored under refrigeration at -20°C. The branches were removed from cold storage and forced with their cut bases in water in a greenhouse under warm temperature and long day conditions for 2, 4 or 6 weeks prior to meristem excision and introduction to in vitro culture. Forcing branches for 2, 4, or 6 weeks resulted in improved survival and growth of mature meristems as compared with non-forced controls. For example, in a recent experiment, survival of meristems from forced branches was 2- to 3-fold higher than for meristems from non-forced branches, 21 weeks post-excision. The 6 weeks forcing treatment had the highest survival of 95%. Forced meristems produced about one new leaf primordium each week while non-forced meristems formed only 3 to 4 primordia overall. Similar results have been obtained for two consecutive years. One of the mature meristems derived shoots produced axillary, brachyblast-like structures in the axils of the scale-like leaves, but they did not elongate into axillary shoots.

Based on the observation that only one of the mature meristem-derived shoots developing from 6 week-greenhouse forced buds had axillary shoots, we hypothesized that scale leaves initiated at this early stage of bud forcing are of the sterile type (Owston, 1969). We have begun to test this hypothesis by culturing meristems from branches collected June 10 which have candles with meristems which have been producing new scale primordia for a few weeks and thus may be past the sterile scale stage. In our initial experiment, meristems from candles at this stage of development have responded very dramatically to a post-excision 3 week treatment with benzylaminopurine (BA) by forming many scale-like leaves. It is still too early to tell whether the scale-like leaves that these meristems have formed have axillary brachyblasts. The challenge now is to improve internode elongation on mature meristem-derived shoots and to obtain axillary shoot development so that we can perform adventitious rooting assays to test for rejuvenation.

Meristem Grafting

A technique for in vitro grafting meristems onto zygotic embryos dissected from imbibed seeds has been developed. With both juvenile and mature meristems as scions, survival of embryo-grafts is greater than with other ex vitro and in vitro grafting techniques we've used. Wounding sites for scions are made on hypocotyls with a 0.3-0.4 mm tissue corer or at the embryo apex by cutting away the meristem and half of the cotyledon primordia with a razor blade splinter. Meristem scions are the same

size as those used for meristem in vitro culture. Grafted embryos are cultured in vitro on Sorbarod supports in liquid half-strength Schenk & Hildebrand media in 3 cm X 10 cm culture vessels under low irradiance fluorescent light at 26°C. With juvenile meristems as scions, survival is 15-25% and there is considerable scion elongation and needle development. Some of the plantlets with juvenile scions have been transplanted to soil. Following decapitation of stock shoots of greenhouse acclimated plants, scions have survived and grown.

Efforts are continuing to perfect grafting techniques using zygotic embryos. Currently, our emphasis is with mature meristems as scions. With the knowledge from excised meristem culture experiments that show that mature meristems have greatest growth potential after greenhouse forcing or natural candle elongation, we will concentrate on using meristems from these developmental stages as scions. We will also do experiments to test for the optimum time (stage of development of the scion) to cut back the stock shoot. Ultimately, we will test for the effect of grafting mature meristems onto juvenile stocks on the maturation characteristics (particularly rooting ability) of resulting shoots.

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